AD_____

Award Number: DAMD17-99-1-9193

TITLE: Characterization of Early Genomic Changes in Mammary Glands of High Risk Women

PRINCIPAL INVESTIGATOR: Bassem R. Haddad, M.D.

CONTRACTING ORGANIZATION: Georgetown University Medical Center
Washington, D.C. 20057

REPORT DATE: July 2002

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release;
Distribution Unlimited

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 074-0188

1. AGENCY USE ONLY (Leave blank)	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	July 2002	Annual (1 Jul 01 - 30 Jun 02)

4. TITLE AND SUBTITLE
Characterization of Early Genomic Changes in Mammary Glands of High Risk Women

5. FUNDING NUMBERS
DAMD17-99-1-9193

6. AUTHOR(S)
Bassem R. Haddad, M.D.

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
Georgetown University Medical Center
Washington, D.C. 20057

E-Mail: haddadb1@georgetown.edu

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSORING / MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES
Report contains color

12a. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

12b. DISTRIBUTION CODE

13. ABSTRACT ***(Maximum 200 Words)***

Because many of the familial breast cancer patients carry a mutation in *BRCA1* on chromosome 17 or *BRCA2* on chromosome 13, the first genetic event that may occur in their mammary glands to begin the progression toward cancer may be loss of heterozygosity (LOH) on one of these two chromosomes. It is unknown if these genetic changes correspond to a recognizable histopathological abnormality. We hypothesize that such genomic changes may precede morphologic changes and thus we may detect evidence for such changes in morphologically normal breast tissues or benign lesions surrounding breast tumors in *BRCA1/2* positive patients. We have recently developed a panel of 15 markers to study LOH in morphologically well characterized and carefully laser capture microdissected, breast tissues from a group of *BRCA1/2* positive patients with breast cancer who are followed up by our Cancer Genetics Program at the Lombardi Cancer Center. Our studies so far support our hypothesis. Specifically, we performed a total of 105 analyses at different loci using microdissected breast tissues from areas showing normal morphology or benign changes surrounding the tumor tissues in *BRCA* carriers with breast cancer. Overall, LOH was detected in 59 studies (56%). In the normal tissues, 15 of 30 analyses (50%) showed LOH and in the tissues with proliferative changes 44 of 75 analyses showed LOH (59%).

14. SUBJECT TERMS	15. NUMBER OF PAGES
Breast cancer, early genomic changes, mammary gland	12
	16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
Unclassified	Unclassified	Unclassified	Unlimited

Table of Contents

Introduction:

Early studies from several groups established that defects on chromosomes 13 and 17 were associated with familial breast cancer. Following the discovery of *BRCA1* on chromosome 17 and *BRCA2* on chromosome 13 (1, 2), more detailed mapping of defects on both chromosomes have been carried out, primarily using LOH methodology. LOH of the two *BRCA* loci has been a highly reproducible observation in tumors of carriers of mutations in each respective gene (3, 4). However, several questions about the actual roles of these two genes in breast tumorigenesis remain unclear. For example, the basis of the vast variation in penetrance of different mutations in these two genes, variations of the same mutation in different individuals of a the same family, and variations among families, are not clear. Our group has a long standing interest in understanding the earliest steps in histopathologic changes and associated genomic alterations as breast cancer begin to arise in high risk, *BRCA*-carrying individuals. However, no studies to date have systematically examined the early consequences of inheritance of a mutation in the *BRCA1* or *BRCA2* genes for corresponding, early changes in breast histopathology. In addition, no studies have addressed the correlation of such early abnormalities in the breasts of *BRCA* mutation carriers with genomic gains, losses, loss of heterozygosity (LOH), or replication error repair instability. Previous studies of both familial and sporadic breast tumors have shown that genetic changes can be detected in morphologically normal appearing breast tissues. Studies of morphologically normal lobules, adjacent to sporadic breast cancer, have shown the presence of LOH in these morphologically "normal" tissues, suggesting the presence of a "field effect" of preexisting genomic damage in the gland, giving rise to the tumor (5). LOH was also detected in hyperplasias (usual ductal hyperplasia and atypical ductal hyperplasia) from both cancerous and noncancerous breasts (6, 7). In addition, two recent studies have also shown cytogenetic abnormalities in prophylactic mastectomy specimens characterized by hyperplasia, without atypia, from patients with a positive family history of breast cancer (but of unknown *BRCA* status) (8, 9). Deletions of 3p14 have been observed in benign proliferative breast disease (9, 10) and one report has shown that the *FHIT* gene was homozygously deleted in two cases of benign proliferative breast disease associated with 3p14 cytogenetic rearrangements and familial breast cancer (11). These studies suggest that loss of the *FHIT* gene may be an early event in mammary carcinogenesis.

Taken together, these studies suggest that there may be detectable early genomic changes in the breasts of high risk patients with inherited predisposition to breast cancer. The purpose of our DOD funded project is to test the hypothesis that genomic changes may be detected not only in hyperplastic, histologically abnormal, premalignant, and malignant regions in the breasts of *BRCA1* and *BRCA2* mutation carriers, with breast cancer, but also in morphologically "normal" looking tissues from *BRCA* carriers with or without breast cancer. Our results support this hypothesis, as we have observed LOH on multiple loci in sclerosing adenosis, in other hyperplastic abnormalities, and in morphologically normal breast tissues from *BRCA1/2* carriers. Such changes may represent the earliest detectable genomic aberrations that occur during the development and progression of breast cancer in these high-risk patients. Identification of these changes will improve our

understanding of the mechanisms of tumorigenesis in these patients, and may be useful to develop molecular markers for early detection and diagnosis of hereditary breast cancer.

Body:

During the third year of this projects, we performed a large number of analyses to complete the main milestones set for this project.

A panel of 15 microsatellite markers (markers for the *BRCA1* gene (intragenic markers and closely linked markers) and for other loci on chromosome 17, closely linked markers to the *BRCA2* gene and other loci on chromosome 13, and intragenic markers for the *FHIT* gene on 3p14.2) was established during the first two years of the project, to evaluate tissue specimens for evidence of LOH. These markers were chosen based on their reported high heterozygosity rate. Prior to the analysis of the LOH in the tumor and adjacent areas, each microsatellite marker from our panel was first evaluated for informativeness using DNA prepared from the patient's peripheral blood. The tumor tissues of the patients and the surrounding areas to the tumor (normal and benign areas) were then evaluated for LOH for the informative markers.

Table 1: The panel of microsatellite markers:

	Site	Size (bp)	Het. (%)
D17S786	17p12	135-157	0.77
TP 53	17p13	230	0.9
D17S849	17p13	215-253	0.67
D17S250	17q11.2-q12	151-169	0.91
D17S806	17q21	153-185	0.91
D17S855	17q21.2 (*BRCA1*)	145	0.82
D17S579	17q21.3	111-133	0.87
D17S785	17q24	181-207	0.84
D17S784	17q25	226-238	0.79
D13S289	13q12.1	260-276	0.74
D13S153	13q14.1-q14.3	212-236	0.82
D13S137	13q14.3	113-135	0.84
D13S173	13q32-q34	166-178	0.84
D3S1300	3p21.1-14.2 (*FHIT*)	217-241	0.83
D3S1481	3p14.2 (*FHIT*)	104	0.83

Using this panel of microsatellite markers, we evaluated a total of 29 areas showing normal morphology (10 areas) or benign changes, such as sclerosing adenosis (19 areas), from four *BRCA1* and one *BRCA2* positive patients with breast cancer. In addition, tumor tissues were also evaluated in each case.

Overall, we performed a total of 105 analyses at different loci; of these, LOH was detected in 59 studies (56%). In the normal tissues, 15 of 30 analyses (50%) showed LOH, and in the tissues with proliferative changes, 44 of 75 analyses showed LOH (59%).
In case 1 (*BRCA1* +), a sequential loss of the *BRCA1* intragenic marker D17S855 was observed in distant areas, up to 8.7mm from the tumor, as well as in the other breast quadrants. An interesting finding was the detection of LOH of this marker in the contralateral breast, removed prophylactically from this patient. In case 3 (*BRCA1* positive), D17S855 showed LOH in the benign areas surrounding the breast tumor, which was remove by lumpectomy. The same marker was also lost in normal tissues and tissues with DCIS in the same breast, removed subsequently by mastectomy. Certain microssatelite markers were altered much more commonly than the others. The markers D17S785 and D17S855 (intragenic to the *BRCA1* gene), demonstrated LOH in the tumor, as well as in the normal/and or benign areas. The D3S1300 marker, intragenic to the *FHIT* gene, was also lost in most of the cases studied, including the *BRCA2* case.

Table 2 summarizes the results:

	LOH found	Total # of analyses
Total	59 (56%)	105
Normal Tissues	15 (50%)	30
Proliferative Changes	44 (59%)	75

Figure 1A shows normal appearing terminal duct lobular units (arrow head) (H&E, 20X) adjacent to the tumor, from a *BRCA1* positive patient with cancer. **Figure 1B**, shows the lobules after laser capture microdissection (LCM) in a consecutive paraffin section. A majority of the epithelial tissue has been microdissected (arrow head). **Figure 1C** shows an area with sclerosing adenosis (arrow head) (H&E, 20X), adjacent to the tumor from the same patient. **Figure 1D** shows LOH analysis using the D17S855 *BRCA1* intragenic marker, performed on tissues microdissected from the same patient's specimens. Tissues studied were isolated using LCM as shown in 1B. From top to bottom, studies in blood show the marker to be heterozygote. Loss of heterozygosity is detected in the tumor. The same allele is also lost in normal lobular tissue adjacent to the tumor (Figure 1A) and in an area with sclerosing adenosis adjacent (Figure 1C) to the tumor. In the contralateral breast, normal tissues do not show LOH, while an area with sclerosing adenosis shows LOH for the *BRCA1* marker.


C

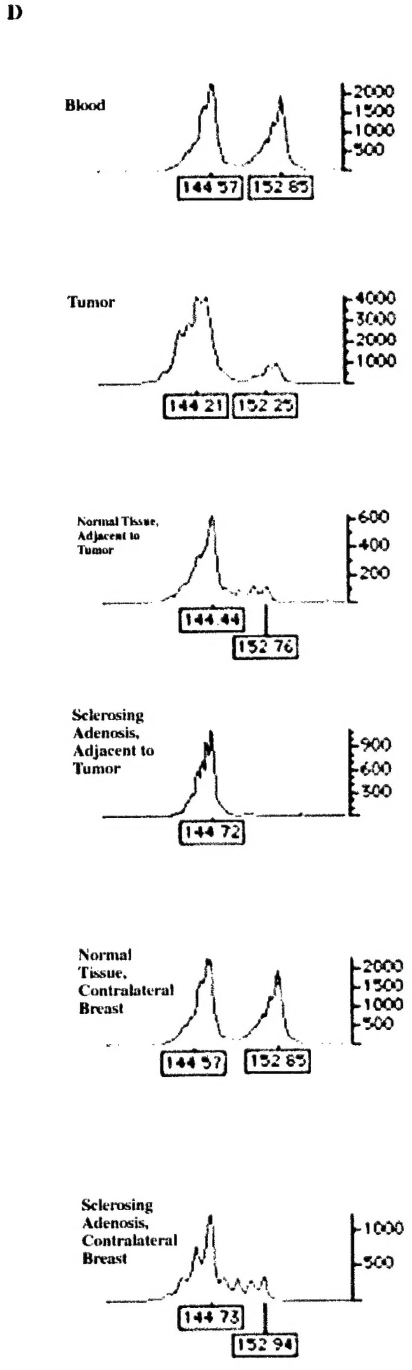
D
Blood
2000
1500
1000
500
144 57
152 85
Tumor
4000
3000
2000
1000
144 21
152 25
Normal Tissue, Adjacent to Tumor
600
400
200
144 44
152 76
Sclerosing Adenosis, Adjacent to Tumor
900
600
300
144 72
Normal Tissue, Contralateral Breast
2000
1500
1000
500
144 57
152 85
Sclerosing Adenosis, Contralateral Breast
1000
500
144 73
152 94

Figure 1

Additional Comments:

Request for a transfer of PI and for a 1 year no cost extension:
In August 2001, we have requested the approval of the DOD for a transfer of PI from Dr. Robert B. Dickson, to the Co-PI Dr. Bassem R. Haddad and for a one year of no cost extension. Both requests have been granted.

Platform Presentation at the American Association for Cancer Research (AACR) 2002 annual meeting:
The work described above was presented in a minisymposium at the 2002 AACR annual meeting (abstract in appendix).

Revised and approved statement of work:

Year 1: In the first year, we will obtain hereditary breast tumors with associated mastectomy tissue as well as prophylactic mastectomies from *BRCA* carriers. [Completed]. We will also fully establish and validate all necessary LOH assays, following pathologic review of all specimens, for comparison of their genomic changes relative to nearby pathologically reviewed and microdissected non-tumor tissue (Aims 1 and 2). [Completed].

Year 2: In the second year, specimen collection will continue, Aims 1 and 2 will continue, and Aims 3 and 4 (study of pathologically reviewed contralateral prophylactic mastectomy tissues and pathologically reviewed bilateral prophylactic mastectomy tissues) will begin. [Completed].

Year 3: In the third year, all 4 aims will be completed and data analyzed. Specifically, pathologic diagnosis will be correlated with genomic and chromosomal changes for each aim. [Work in progress]

Key Research Accomplishments:

-Development of a panel of microsatellite markers to study LOH on chromosomes 13, 17 and 3p in laser capture microdissected (LCM) specimens.
-LOH analysis of morphologically normal breast tissues and breast tissues with benign changes, carefully microdissected from *BRCA1/2* positive patients with breast cancer.

Reportable Outcomes:

Cavalli, LR., Singh, B., Isaacs C., Dickson RB., and Haddad, BR. Evidence of genomic instability in morphologically normal breast tissues and in benign breast lesions in *BRCA1/2* positive patients with breast cancer. *Manuscript in preparation.*

Conclusion:

Our data support our hypothesis, namely that genetic changes may indeed occur in morphologically normal breast tissues, or breast tissues showing benign changes in *BRCA1/2* positive patients with breast cancer. These findings might lead to the identification of genetic markers that could be used to assist in the early detection of breast cancer in women at risk. During the final year of the project we will finalize the analysis of the data and correlate the genomic and chromosomal changes with the pathologic findings. We will also complete the manuscript which describes this work and present our final report to the DOD.

References:

1. Miki Y, Swensen J, Shattuck-Eidens D, Futreal PA, Harshman K, Tavtigian S, Liu Q, Cochran C, Bennett LM, Ding W: A strong candidate for the breast and ovarian cancer susceptibility gene BRCA1. Science 266:66-71, 1994
2. Wooster R, Bignell G, Lancaster J, Swift S, Seal S, Mangion J, Collins N, Gregory S, Gumbs C, Micklem G: Identification of the breast cancer susceptibility gene BRCA2. Nature 378:789-92., 1995
3. Cornelis RS, Neuhausen SL, Johansson O, Arason A, Kelsell D, Ponder BA, Tonin P, Hamann U, Lindblom A, Lalle P, et al.: High allele loss rates at 17q12-q21 in breast and ovarian tumors from BRCAl-linked families. The Breast Cancer Linkage Consortium. Genes Chromosomes Cancer 13:203-10., 1995
4. Gudmundsson J, Johannesdottir G, Arason A, Bergthorsson JT, Ingvarsson S, Egilsson V, Barkardottir RB: Frequent occurrence of BRCA2 linkage in Icelandic breast cancer families and segregation of a common BRCA2 haplotype. Am J Hum Genet 58:749-56., 1996
5. Deng G, Lu Y, Zlotnikov G, Thor AD, Smith HS: Loss of heterozygosity in normal tissue adjacent to breast carcinomas. Science 274:2057-9., 1996
6. O'Connell P, Pekkel V, Fuqua S, Osborne CK, Allred DC: Molecular genetic studies of early breast cancer evolution. Breast Cancer Res Treat 32:5-12, 1994
7. O'Connell P, Pekkel V, Fuqua SA, Osborne CK, Clark GM, Allred DC: Analysis of loss of heterozygosity in 399 premalignant breast lesions at 15 genetic loci. J Natl Cancer Inst 90:697-703, 1998
8. Teixeira MR, Pandis N, Gerdes AM, Dietrich CU, Bardi G, Andersen JA, Graversen HP, Mitelman F, Heim S: Cytogenetic abnormalities in an in situ ductal carcinoma and five prophylactically removed breasts from members of a family with hereditary breast cancer. Breast Cancer Res Treat 38:177-82, 1996
9. Petersson C, Pandis N, Mertens F, Adeyinka A, Ingvar C, Ringberg A, Idvall I, Bondeson L, Borg A, Olsson H, Kristoffersson U, Mitelman F: Chromosome aberrations in prophylactic mastectomies from women belonging to breast cancer families. Genes Chromosomes Cancer 16:185-8., 1996
10. Dietrich CU, Pandis N, Teixeira MR, Bardi G, Gerdes AM, Andersen JA, Heim S: Chromosome abnormalities in benign hyperproliferative disorders of epithelial and stromal breast tissue. Int J Cancer 60:49-53., 1995
11. Panagopoulos I, Pandis N, Thelin S, Petersson C, Mertens F, Borg A, Kristoffersson U, Mitelman F, Aman P: The FHIT and PTPRG genes are deleted in benign proliferative breast disease associated with familial breast cancer and cytogenetic rearrangements of chromosome band 3p14. Cancer Res 56:4871-5., 1996

Appendix:

Abstract presented at the AACR 2002 Annual Meeting.

Evidence for Genomic Instability in Morphologically Normal Breast Tissues and in Benign Breast Lesions in *BRCA1/2* Positive Patients with Breast Cancer. Cavalli, LR., Singh, B., Isaacs C., Dickson RB., and Haddad, BR.

Because many of hereditary breast cancer patients carry a mutation in *BRCA1* on chromosome 17 or *BRCA2* on chromosome 13, the first genetic event that may occur in their mammary glands to begin the carcinogenic progression toward cancer may be loss of heterozygosity (LOH) on one of these two chromosomes. It is unknown if these genetic changes correspond to a recognizable histopathological abnormality, or at what stage of the disease progression they occur. In this study, we evaluated the early genomic changes that occur in the mammary glands of patients with increased predisposition to breast cancer due to germ-line mutations in the *BRCA1/2* genes. We tested the hypothesis that these genomic changes may be detected not only in the histologically abnormal and malignant regions of these high risk women, but also in morphologically normal looking tissues and in areas with benign changes. Using a panel of 15 microsatellite markers we have evaluated 23 areas showing normal morphology or benign changes, such as sclerosing adenosis, from four *BRCA1* and one *BRCA2* positive patients with breast cancer. For each case, tissue sections were carefully evaluated by the pathologist and areas with morphologically normal tissues surrounding the tumor and areas with benign changes such as sclerosing adenosis were marked and microdissected using Laser Capture Microdissection (LCM) technology prior to LOH analysis. LOH was detected in both morphologically normal tissues and tissues with benign changes from all the cases investigated. Allelic losses of a minimum of 2 different markers, were observed in 17 of 23 areas with normal morphology or benign changes (Sclerosing Adenosis). In a *BRCA1* case, a sequential loss of two markers on chromosome 17q, previously observed in the tumor, was observed in morphologically normal looking areas up to 8mm distant from the tumor, as well, as in the other breast quadrants from the same breast where the tumor was present. An interesting finding was the detection of LOH in the normal contralateral breast removed prophylactically from this patient. Such changes may represent the earliest detectable genomic aberrations that occur during the development and progression of breast cancer in these high-risk patients. These data will aid in improved early detection and diagnosis of hereditary breast cancer and provide more information when considering prevention strategies for such women at risk.